

In re Application of: Eilon Barnea
Serial No.: 10/705,459
Filed: November 12, 2003
Office Action Mailing Date: February 23, 2007

2

Examiner: Dibrino, Marianne NMN
Group Art Unit: 1644
Attorney Docket: 26884

AMENDMENTS TO THE SPECIFICATION

In the Specification:

Please replace the paragraph on page 27, lines 15-18 which states:

"There are several thousands of MHC genes, some of which were cloned. Table 5 below associates the MHC genes into classes and types (6). The sequences of the known MHC genes can be found in the Kabat database (<http://immuno.bme.nwu.edu/>)."

With

"There are several thousands of MHC genes, some of which were cloned. Table 5 below associates the MHC genes into classes and types (6). The sequences of the known MHC genes can be found in the Kabat database <http://immuno dot bme dot nwu dot edu/>"

Please replace the paragraph on page 51, lines 5-25 which states:

"The MHC bound peptides were resolved by reverse-phase HPLC on a 0.1 ID fused silica capillaries with length of about 30 cm (J&W) slurry packed with POROS 10 R2 (PerSeptive Biosystems). The capillaries were fitted with electrospray needle made from 36-gauge stainless tubing (Small Parts Inc. Miami Lakes, FL). A Rheodyne 9125 HPLC injector fitted with a 20 μ l loop was used for loading the column. The peptides were resolved by a relatively long (90 minutes) linear gradient of 5 to 50 % acetonitrile with 0.1 % acetic acid, at a flow rate of about 1 μ l/minute. The flow was electrosprayed directly from the HPLC column into an ion trap mass spectrometer (LCQ, Finnigan). The mass spectrometry analysis was done in the positive ion mode, using repetitively a full MS scan usually between 450 to 1500 atomic mass units (amu) followed by collision-induced decomposition (CID) of the dominant ion selected from the previous MS scan. In some cases the full MS was performed with a narrower mass range to reduce the number of detected peptides. The peptides were identified by comparing their MS

and CID data to the calculated MS and CID of the proteins in the Genpept databank (www.ncbi.nlm.nih.gov/genpept) using the Sequest software [25] (obtained from Finnigan, San Jose, CA). The number of times each peptide was fragmented by CID was usually limited to two by dynamic exclusion, a feature of the Xcalibur control software the LCQ mass spectrometer (Finnigan)".

With

"The MHC bound peptides were resolved by reverse-phase HPLC on a 0.1 ID fused silica capillaries with length of about 30 cm (J&W) slurry packed with POROS 10 R2 (PerSepetive Biosystems). The capillaries were fitted with electrospray needle made from 36-gauge stainless tubing (Small Parts Inc. Miami Lakes, FL). A Rheodyne 9125 HPLC injector fitted with a 20 μ l loop was used for loading the column. The peptides were resolved by a relatively long (90 minutes) linear gradient of 5 to 50 % acetonitrile with 0.1 % acetic acid, at a flow rate of about 1 μ l/minute. The flow was electrosprayed directly from the HPLC column into an ion trap mass spectrometer (LCQ, Finnigan). The mass spectrometry analysis was done in the positive ion mode, using repetitively a full MS scan usually between 450 to 1500 atomic mass units (amu) followed by collision-induced decomposition (CID) of the dominant ion selected from the previous MS scan. In some cases the full MS was performed with a narrower mass range to reduce the number of detected peptides. The peptides were identified by comparing their MS and CID data to the calculated MS and CID of the proteins in the Genpept databank (www.ncbi.nlm.nih.gov/genpept) using the Sequest software [25] (obtained from Finnigan, San Jose, CA). The number of times each peptide was fragmented by CID was usually limited to two by dynamic exclusion, a feature of the Xcalibur control software the LCQ mass spectrometer (Finnigan)".

In re Application of: Eilon Barnea
Serial No.: 10/705,459
Filed: November 12, 2003
Office Action Mailing Date: February 23, 2007

4

Examiner: Dibrino, Marianne NMN
Group Art Unit: 1644
Attorney Docket: 26884

Please replace the paragraph on page 50, lines 1-5 which states:

"The hybridomas W6/32 and BB7.2, an anti-MHC class-I and anti-HLA-A2 respectively, and HB-149 an anti β 2m were obtained from the ATCC. The antibodies were affinity purified using protein A-Sepharose CL-4B (Sigma) from mouse ascites fluid".

With

"The hybridomas W6/32 and BB7.2, an anti-MHC class-I and anti-HLA-A2 respectively, and HB-149 an anti β 2m were obtained from the ATCC. The antibodies were affinity purified using protein A-SEPHAROSE™ (agarose) CL-4B (Sigma) from mouse ascites fluid".

Please replace the paragraph on page 50, lines 18-29 which states:

Cultured cells, expressing the soluble MHC were grown to confluence in 150 mm plates. The culture medium was collected and residual cells were removed by centrifugation. Soluble MHC molecules were purified from the cleared culture medium by affinity chromatography on W6/32 antibody columns at 4 °C. The antibodies were coupled to NHS-activated agarose (Pharmacia) or to protein A Sepharose (Sigma) with n-methylpipelimidate (Sigma). The columns were washed with 0.5 M NaCl, 20 mM, Tris pH 8. The MHC molecules were eluted from the column with 0.1 M acetic acid at pH 3. Peptides were separated from the MHC complexes by boiling for five minutes in 10 % acetic acid followed by ultra-filtration through a 3 kDa Microcon (Amincon) [2].

With

Cultured cells, expressing the soluble MHC were grown to confluence in 150 mm plates. The culture medium was collected and residual cells were removed by centrifugation. Soluble MHC molecules

In re Application of: Eilon Barnea
Serial No.: 10/705,459
Filed: November 12, 2003
Office Action Mailing Date: February 23, 2007

5

Examiner: Dibrino, Marianne NMN
Group Art Unit: 1644
Attorney Docket: 26884

were purified from the cleared culture medium by affinity chromatography on W6/32 antibody columns at 4 °C. The antibodies were coupled to NHS-activated agarose (Pharmacia) or to protein A SEPHAROSE™ (agarose) (Sigma) with n-methylpipelimide (Sigma). The columns were washed with 0.5 M NaCl, 20 mM, Tris pH 8. The MHC molecules were eluted from the column with 0.1 M acetic acid at pH 3. Peptides were separated from the MHC complexes by boiling for five minutes in 10 % acetic acid followed by ultra-filtration through a 3 kDa Microcon (Amincon) [2].

Please replace the paragraph on page 11, lines 19-25 which states:

FIGs. 2A-C demonstrate the purification process of soluble HLA-Cw4 from human ovarian cancer cells. (2A) purification of sHLA-Cw4 from the growth medium of UCI-101 transfected cells. Column S, represents first purification step on sepharose beads. Column W6, represents second purification step by affinity chromatography on W6/32 antibody column; (2B) SDS-PAGE analysis and Coomassie staining of eluted fractions (e1-e5); (2C) Western blot analysis of eluted fractions.

With

FIGs. 2A-C demonstrate the purification process of soluble HLA-Cw4 from human ovarian cancer cells. (2A) Chromatography purification of sHLA-Cw4 from the growth medium of UCI-101 transfected cells. Column S, represents first purification step on SEPHAROSE™ (agarose) beads. Column W6, represents second purification step by affinity chromatography on W6/32 antibody column; (2B) SDS-PAGE analysis and Coomassie staining of eluted fractions (e1-e5); (2C) Western blot analysis of eluted fractions.

In re Application of: Eilon Barnea
Serial No.: 10/705,459
Filed: November 12, 2003
Office Action Mailing Date: February 23, 2007

6

Examiner: Dibrino, Marianne NMN
Group Art Unit: 1644
Attorney Docket: 26884

Please replace the paragraph on page 1, lines 10-15 which states:

"This application is a continuation in part of PCT Patent Application No. PCT/IL02/00383, filed May 16, 2002, which claims priority from pending U.S. Patent Application No. 09/865,548, filed May 29, 2001, and U.S. Provisional Application No. 60/290,958, filed May 16, 2001"

With

"This application is a continuation in part of PCT Patent Application No. PCT/IL02/00383, filed May 16, 2002, which claims priority from pending U.S. Patent Application No. 09/865,548, filed May 29, 2001, now US Patent No. 6,867,283 and U.S. Provisional Application No. 60/290,958, filed May 16, 2001"

Please replace the paragraph on page 88, lines 1- which states:

"S=79 (85, 66) Mp=1194.5 Tp=49 B7=360/31
P=APSGSLAVPLAVL genpept
PR=>gi|5262492|emb|CAB45700.1| (AL080080)
hypothetical protein [Homo sapiens] POS=9 (SEQ ID
NO: 37) "

With

"S=79 (85, 66) Mp=1194.5 Tp=49 B7=360/31
P=APSGSLAVPLAVL genpept
PR=>gi|5262492|emb|CAB45700.1| (AL080080)
hypothetical protein [Homo sapiens] POS=9 (SEQ ID
NO: 37) "